content at the higher concentration was 14.1 ± 2.5 nmole/g ($N = 6$, $P < .01$). The ambient glucose concentration thus appears to regulate the sorbitol content of the aortic wall.

Aldose reductase was isolated from rabbit thoracic aorta and from fresh autopsy specimens of human thoracic aorta. Frozen tissue (200 g) from 400 rabbits or pooled human specimens was minced and homogenized in a Virtis tissue homogenizer at 2°C in phosphate buffer (5 mM), pH 6.8, containing 2-mercaptoethanol (1 mM). The homogenates were centrifuged at 20,000g for 30 minutes at 2°C. The protein content of the supernatant was determined (*5*), and twice the dry weight of aged calcium phosphate gel was added. After centrifugation, the supernatant was subjected to ammonium sulfate fractionation. The protein precipitating in the 40 to 80 percent saturated ammonium sulfate fraction was dissolved in a small volume of phosphate buffer (5 mM), pH 6.8, and passed through a column of Sephadex G-50 that had been equilibrated with the same buffer. The protein was then applied to a column (2.5 by 25 cm) of diethylaminoethylcellulose (DEAE) equilibrated with phosphate buffer (5 mM), pH 6.8. The column was washed with 200 ml of the same buffer and developed with 750 ml of a linear gradient of phosphate buffer, pH 6.8, which increased from 5 mM to 25 mM.

Two peaks of nicotinamide adenine dinucleotide phosphate (NADP) polyol dehydrogenase activity were recovered. The first peak, which had the characteristics of aldose reductase, eluted between 16 and 19 mM of phosphate. When assayed in phosphate buffer (67 mM), pH 6.2, aldose reductase from rabbit aorta had the following K_m's: with D-xylose as substrate, 29 mM; with D-glucose, 130 mM; with potassium D-glucuronate, 28 mM; and with D-glucuronolactone, 2 mM. The K_m with NADPH as substrate was 0.015 mM, and no activity was observed with NADH. The specific activity of the rabbit aortic aldose reductase was 72 international units per gram. The aldose reductase preparations used in these studies were subsequently subjected to column chromatography on a column (2.5 by 80 cm) of Sephadex G-100 equilibrated with phosphate buffer (5 mM), pH 6.8, and developed with the same buffer. Aldose reductase activity was associated with a single peak of protein. The K_m's of aldose reductase isolated from human aorta were, with xylose as substrate, 10 mM; with glucose, 180 mM; with glucuronate, 70 mM; with glucuronolactone 2 mM; and with NADPH, 0.007 mM. The comparable K_m values for aldose reductase isolated from rabbit lens by the same technique were for xylose, 69 mM; glucose, 300 mM; glucuronate, 33 mM; glucuronolactone, 6 mM; and NADPH, 0.04 mM. The second peak of NADP polyol dehydrogenase activity eluted from the DEAE-cellulose column had the characteristics of L-gulonate: NADP oxidoreductase (E.C. 1.1.1.19) with a K_m glucose in excess of 2M for both the human and rabbit aortic enzymes.

Activity of sorbitol dehydrogenase was demonstrated in the supernatant fraction of rabbit thoracic aortic homogenates (1:1, weight: volume; 0.25M sucrose; centrifuged at 100,000g for 30 minutes at 2°C). Activity was assayed fluorometrically in 1.0 ml of glycine–NaOH buffer (50 mM), pH 9.6, containing NAD (0.4 mM) and sorbitol (50 mM). The initial velocity was linearly related to the volume of supernatant added over the range of 0.005 to 0.015 ml. In four such experiments the rates of sorbitol oxidation were 11.3, 13.3, 15.2, and 10.2 nmole min^{-1} g^{-1} (wet weight). Both enzymes of the polyol pathway are therefore present in the aorta.

L-Epinephrine added in vitro increased the aortic sorbitol content of paired aortic samples incubated with glucose (5 mM) and produced linear increments over the range of 0.5 to 5.0 μg/ml (Figs. 1 and 2). This effect was reproduced by the beta receptor stimulator isoproterenol, but not by the alpha receptor stimulator norepinephrine (Fig. 2). Dibutyryl-3′,5′-adenosine monophosphate also increased aortic sorbitol content when added in vitro (Fig. 2). In addition, it was observed that ouabain and angiotensin II increased the sorbitol content of aortic tissue incubated in vitro with glucose (5 mM) (Fig. 2). In the same system, prostaglandins E_1, E_2, and $F_{1\alpha}$ had no significant effect when added in concentrations of 1 μg/ml. Moreover, these prostaglandins did not inhibit the effect of epinephrine (2 μg/ml) added in vitro.

The mechanism by which these agents produce their effects on aortic sorbital content has not been clearly established. Since glucose-6-phosphatase is not present in the aortic wall (*4*), increased phosphorylase activity cannot materially increase the free intracellular glucose concentration. The effect of epinephrine on aortic sorbitol content can be demonstrated in the absence of oxygen.

The demonstration of the polyol pathway in the aortic wall provides the first evidence of a mechanism by which hyperglycemia can directly alter the metabolism of the arterial wall. In addition, these hormonal effects raise the more fundamental question of the normal function of aldose reductase in tissues other than the seminal vesicle.

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Centromeres in Human Meiotic Chromosomes

Abstract. *Two heterochromatic spots were observed in most bivalents of human spermatocytes at the late diplotene stage from six individuals. It is our belief that these dark staining bodies are the centromeres of the meiotic chromosomes.*

The size of the chromosome and position of the centromere are the basis for mitotic chromosome karyotyping (*1*). Recently we suggested a similar procedure for karyotyping meiotic chromosomes according to the size of the bivalents (*2*). At that time we were not able to locate the centromere.

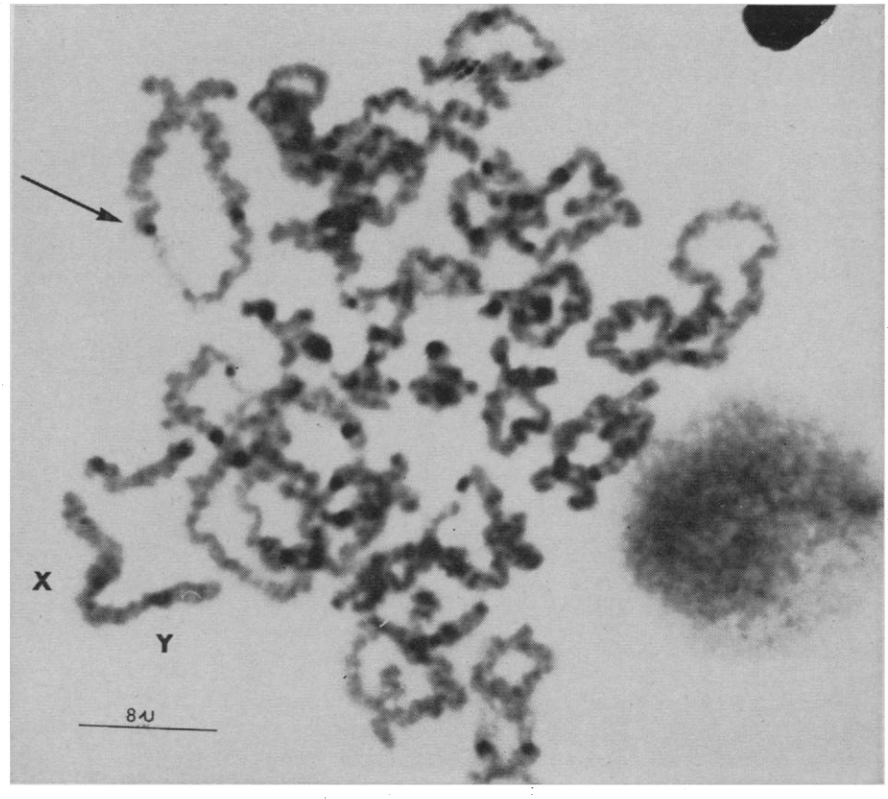
Modification of techniques described previously (*3*) permitted clear and permanent preparation of our present material. The critical difference in our procedure is the use of Giemsa instead of aceto-orcein stain.

Our specimens, provided by urologists in the Atlanta area, were derived from six normal-appearing men, two treated for hydrocele, three for cancer of the prostate, and one for epididymitis. This report is based on an analysis of spermatocytes in the late diplotene stage of the first meiotic division. A minimum of ten cells was examined in each of two subjects and over 30 cells from each of the other four individuals. In most of the bivalents examined, we observed two clearly defined round black staining bodies (Fig. 1).

We believe these heterochromatic spots are the centromeres of the meiotic chromosomes at the late diplotene stage because (i) in other species, centromeres in meiotic chromosomes have been described as dark staining bodies (*4–7*), (ii) on theoretical grounds it is expected that there should be two bodies in each bivalent, and this was the case, (iii) the pair of black spots occurred at corresponding positions in each half bivalent and were separated from each other, and (iv) the locations of these dark bodies in each bivalent (Fig. 2) were comparable with the similar position of the centromeres in mitotic metaphase.

The role of centromeres in meiosis and mitosis is well known and is consistent throughout the plant and animal kingdom. The centromere in somatic chromosomes of many organisms appears simply as a nonstaining constriction with no morphological evidence of structure (*8*). Most studies of centromere structure in meiotic chromosomes were conducted in plants; results of these investigations varied. McClintock believed that the centromeres of the pachytene chromosomes in maize were nonstainable structureless ovoid bodies (*9*). The meiotic metaphase centromeres in *Tradescantia* were seen as small stainable granules (*4*). The pachytene chromosomes of *Secale* and *Agapanthus* were reported as compound structures with granules which varied in size (*10*).

In vertebrates, meiotic chromosomes of the amphibian *Amphiuma* (*5*) were stained with iron hematoxylin, those of the mouse (*6*) with Sudan black B, and those of the Wallaby kangaroo (*7*) with aceto-orcein. All were described as showing dark staining bodies, interpreted as centromeres. The human testicular material which we stained with Giemsa also revealed dark staining bodies which we consider to be the centromeres. This is the first time that dark staining centromeres of meiotic chromosomes at the diplotene stage have been observed in man.



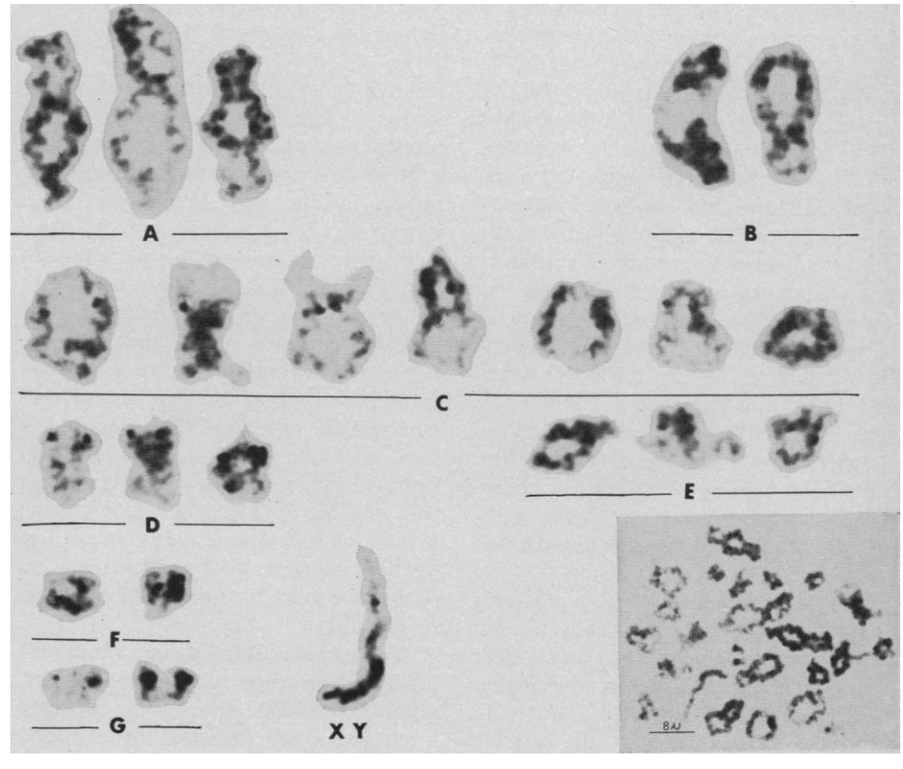


Fig. 1 (top right). Centromere positions in human male meiotic bivalents. Fig. 2 (bottom right). Karyotyping of human male meiotic chromosomes according to size and centromere position.

In order to further evaluate our observations, the meiotic tissue was also stained with aceto-orcein, iron hematoxylin, and Sudan black B. In our preparations aceto-orcein deeply stained the meiotic chromosomes and obscured the centromeres, while preliminary results with iron hematoxylin and Sudan black B indicated that these dark staining bodies were present in each bivalent.

If the heterochromatic bodies observed in our preparations are indeed centromeres, then cytological evidence is available with regard to the XY association of man in the late diplotene stage. The XY bivalents (Figs. 1 and 2) reveal that the short arm of the Y chromosome is in association with the short arm of the X chromosome. This is a consistent finding in all of our material.

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Sex Pheromones Produced by Male Boll Weevil: Isolation, Identification, and Synthesis

Abstract. *The response of female boll weevils to males,* Anthonomus grandis *Boheman, in laboratory bioassays can be reproduced by exposure to a mixture of compounds I, II, and either III or IV, all isolated from male weevils and their fecal material. The same response was elicited by mixtures of the synthesized compounds. Compound I is* (+)-cis-*2-isopropenyl-1-methylcyclobutaneethanol; II,* cis-*3,3-dimethyl-$\Delta^{1,\beta}$-cyclohexaneethanol; III,* cis-*3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde; and IV,* trans-*3,3-dimethyl-$\Delta^{1,\alpha}$cyclohexaneacetaldehyde.*

We have isolated and identified two terpene alcohols and two terpene aldehydes from male boll weevils, *Anthonomus grandis* Boheman, and their fecal material. These compounds are the components of the pheromone to which only female boll weevils respond in laboratory tests. They may also be aggregating pheromones, because live males attract only females in the laboratory, but attract both sexes in the field in the spring and fall (*1*). The active compounds, each of which has been synthesized, are shown in Fig. 1. The success in isolating, identifying, and synthesizing the pheromone may point the way to new methods of surveying and controlling the boll weevil (*1*).

In laboratory bioassays (*1*), mixtures containing all four compounds elicited a response by females equivalent to or better than that elicited by live males. Absence of either alcohol (I, II) or both aldehydes (III, IV) from the mixture resulted in almost complete lack of response (Table 1). The response to mixtures of synthetic compounds was identical to that obtained from corresponding mixtures of the natural compounds. The synergistic effect observed with these four compounds is similar to, although slightly more complex than, that observed for the sex pheromone of the male *Ips confusus* (Le Conte) (*2*).

The four compounds were isolated from insects (67,000 males and 4.5 million weevils of mixed sexes) and from fecal material (54.7 kg) in identical fashion. No masking of the activity was evident in material derived from weevils of mixed sexes after steam distillation. Neither males nor females were responsive to extracts or steam distillates of females.

In brief, the isolation procedure (*3*) involves extraction of the source material, steam distillation, and subsequent fractionation by column chromatography on Carbowax 20M-coated (*4*) silica gel eluted successively with pentane, pentane–ether (90 : 10), pentane–ether (50 : 50), ether, and methanol. None of the individual fractions from this column were active, but the combination of fractions two and three, eluted with pentane–ether (90 : 10) and (50 : 50), respectively, was as active as the original distillate. Each of these fractions was then further fractionated on a column containing Adsorbosil-CABN (25 percent $AgNO_3$ on silica gel), which was eluted with the same series of solvents.

The material eluted with pentane–ether (90 : 10) from Carbowax 20M/silica gel and pentane–ether (50 : 50) from $AgNO_3$-silica gel gave six peaks on gas-liquid chromatography (GLC) on Carbowax 4000. The sixth peak, whose Kovats index [I_k (*5*)] was 1782 on Carbowax 4000, and which appeared homogeneous on the SE-30 column ($I_k = 1248$) gave two peaks, compounds III and IV, on a 15.2-m SCOT column coated with Carbowax 20M.

The material eluted with pentane–ether (50 : 50) from both liquid columns gave eight peaks on GLC on Carbowax 4000. Peak eight ($I_k = 1820$ on Carbowax 4000) gave two peaks, compounds I and II ($I_k = 1205$ and 1228, respectively), on the SE-30 column.

Compounds I and II were collected individually for bioassay by bubbling the effluent from the SE-30 column through dichloromethane. Compounds III and IV, combined in a single peak, were similarly collected from the SE-30 column. For the spectral studies, collections were made in carbon tetrachloride.

The concentrations of compounds I and II in fecal material were determined by combining a weighed amount of *p*-menth-1-en-4-ol (terpinen-4-ol) with the fraction eluted from both liquid columns with pentane–ether (50:50), by gas chromatography of the mixture, and by determination of the relative peak areas of the standard and the two natural products. Compounds I and II were thus calculated to be present in fecal material in concentrations of 0.76 and 0.57 ppm, respectively. Similarly, with 3,7-dimethyl-6-octenal (citronellal) as an internal standard, compounds III and IV were each found to constitute 0.06 ppm of fecal material. Concentrations in mixed weevils were about tenfold less.

The identity of compound I was determined on the basis of its mass, nuclear magnetic resonance (NMR), and